

SEM Analysis of Residual Dentin Surface in Primary Teeth Using Different Chemomechanical Caries Removal Agents

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Background: The purpose of this *in vitro* study was to analyze the residual dentinal surfaces following caries removal using two chemomechanical methods (Papacarie Duo and Carie Care), by scanning electron microscopy (SEM). **Study design:** Twenty extracted primary molars with active occlusal carious lesions were randomly assigned two groups depending on the CMCR agent used for the caries excavation – Group 1 – with Papacarie Duo and Group – 2 with Carie Care. After the caries excavation, the specimens were subjected to SEM analysis. **Results:** Though both the agents showed the minimal smear layer with the patent dentinal tubules, Carie care showed patent dentinal tubules with a clearly exposed peritubular and intertubular collagen network. **Conclusion:** Carie Care treated surface exhibited better surface morphology of residual dentin.

Key words: SEM, residual dentin, chemomechanical caries removal, Carie care, primary teeth

INTRODUCTION

Dentin substrate is a vital hydrated composite material with structural components and properties that may be altered by physiological processes, age and diseases. Significant variations in its architecture may occur according to the depth and response to previous injuries such as carious lesions and cavity preparations^{1,2}. With the advent of the adhesive restorative materials, the concept of caries excavation with preservation of healthy dentin has gained popularity. In order to accomplish this principle, the procedures available include: air abrasion with aluminum oxide, CMCR, atraumatic restorative therapy (ART) and most recently, lasers³. The CMCR method stands out among the other minimal invasive options as it address the advantages like cost

effectiveness, patient's compliance, avoid the use of local anesthesia, preserve the healthy dentin intact and facilitate ultimate tissue preservation⁴. The CMCR system involves selective dissolution of outermost portion (infected layer), by the application of a natural or synthetic agent, followed by atraumatic mechanical removal leaving behind the affected demineralized dentin that can be remineralized and repaired⁵.

Considering the advantages of CMCR, a gel based on papain containing chloramine and toluidine blue named Papacarie DuoTM was developed in Brazil in 2003. A number of studies can be found in the literature evaluating the clinical efficacy and surface topography of residual dentin and remnant tooth structure with this material after caries removal^{2,5-8}. The data corroborated, indicated Papacarie DuoTM to be a clinically efficient material in terms of the dentin surface morphology and non-interference with the resin composite bonding^{2,9,10}.

An alternative to Papacarie DuoTM, named Carie CareTM was launched in India in 2011 with an intent to be more cost effective, which is composed of papain, clove oil, and chloramine⁹. This gel is based on papaya extract, an endoprotein rich in basic amino acids in combination with essential therapeutic oils. The active ingredient has proteolytic action that would soften the pre degraded collagen of the lesion without pain or any undesirable effects on adjacent healthy tissues with added antiseptic and anti-inflammatory properties of the essential therapeutic oils^{11,12}.

Due to the difference in composition, the two materials will yield a different pattern of dentin substrate. Therefore giving the relevance to the subject, the emergence of new CMCR material and the scarcity of the studies in this regard on primary teeth, the present study aimed at evaluating remaining dentinal substrate after caries removal in primary teeth using two different CMCR agents—Carie CareTM and Papacarie DuoTM using SEM.

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MATERIALS AND METHOD

Twenty recently extracted human primary molars with an active occlusal carious lesion and internal or external root resorption indicated for extraction from the out patients visiting the Department of Pedodontics and Preventive Dentistry, K.L E Society's Institute of Dental Sciences, Bangalore were included for the study. The teeth were extracted, collected and stored as per the occupational safety and health regulations¹³. The informed consent was obtained from all participating parents or legal guardians of minors for using the extracted teeth for the research purpose. The study was approved by the institutional research ethics committee prior to the commencement. Each tooth had occlusal caries to a depth of approximately 1-2mm below the central fissure, as assessed with an exploratory probe. Immediately after the extraction, the teeth were stored in deionized water and were evaluated within seven days. All the teeth were cleaned with prophylactic instruments and were randomly divided into two basic groups according to the method of caries removal for evaluation of ultrastructure of residual dentin.

Group I—consisted of 10 samples prepared after caries removal using Papacarie Duo™ (Formula & Acao, Sao Paulo SP, Brazil).

Group II—consisted of 10 samples prepared using Carie Care™ (Innovation - Hub 5, Bangalore, India).

The caries removal was performed using either of the two methods according to the manufacturer's instructions. The gel was applied on to the carious lesion and left undisturbed for 30- 60 seconds. The softened carious dentin was then scraped using a blunt excavator (EXC 245, Hu-Friedy Mfg. Co., LLC, Chicago). The procedure was repeated 2-3 times on an average and caries removal was ceased when the gel attained a non-turbid appearance, which was considered caries free. The residual gel was then removed and the cavity was wiped with a moistened cotton pellet and rinsed. The complete procedure of caries excavation required a mean time of 6-8 minutes. The visual and the tactile criteria were followed for confirmation of the complete removal of active carious lesion. The visual method of assessment of caries removal was based on non-turbid appearance and unchanged light color of the CMCR agent used for caries excavation. The tactile assessment of sound dentin was gauged by the test performed with an exploratory probe¹⁴.

Sample preparation for SEM viewing

The specimens of both groups were subjected to an ultrasonic bath for 5 minutes in sterile distilled water. Subsequently they were dried overnight in a desiccator until all residual moisture had been removed. Then the prepared samples were sputtered coated (Sputter coater, Q150T S, Quorum technologies Ltd, United Kingdom) and introduced into vacuum chamber of SEM (FE-SEM, Carl Zeiss Neon 40 Crossbeam, Germany). A series of micro-photographs were taken at a magnification of x5000 and x10,000 for viewing the surface morphology.

RESULTS

Differences were noticed between the dentin surfaces obtained after using the two different caries removal systems. The images of dentin after caries removal in Group-I are shown in Figures-1 and 2. The morphological details of remaining dentinal substrate in Group-I exhibited two different patterns of remaining dentin. A very irregular and rough surface covered by an amorphous layer indicating the presence of smear layer, obliterating the dentinal tubules and few patent dentinal tubules (Figure - 1). The dentinal tubules were patent, inorganic dentin debris were detected with little smear layer and exposed dentinal tubules. (Figure - 2)

In Group II, a substantial portion of dentin surface was free from smear layer. The dentin surface possessed intertubular microporosity. The surface morphology showed patent dentinal tubules with a clearly exposed peritubular and intertubular collagen network. The exposed superficial intertubular collagen network is obviously different from that of the normal dentin sample. The distinctly reticular collagen fiber network with a random orientation was noted (Figure - 3). The dentinal tubules were open in most locations due to a minimal smear layer. Some residual smear layer and a fibrous structure inside the tubules were revealed. Partial opening of dentinal tubules with a residual mineral deposit was demonstrated (Figure - 4). A noticeable smear layer and occluded dentinal tubules remained on the residual dentin surface were present in some areas (Figure - 5).

DISCUSSION

It has been reported that chemo-mechanically treated dentin has a higher surface energy than conventionally treated dentin. This implies that the chemo-mechanically treated dentin may have greater affinity for adhesive materials and better bonding than conventionally treated dentin¹⁵. Thus the present study was undertaken to assess the ultramorphology of residual dentin in primary teeth after caries excavation with two CMCR agents. The result of this study supports our hypothesis and suggests that samples prepared using Carie Care™ exhibited better surface morphology than that obtained with Papacarie Duo™.

In order to accomplish the objective of biomimetic dental reconstruction and maintain the pulpal vitality after restoration, it is important to evaluate the state of residual dentin after excavation of caries¹⁶. Using histologic, biochemical, biomechanical, microscopic and microbiologic techniques, researchers were able to distinguish two layers in caries lesions that were very different in nature i.e. outer carious dentin and inner carious dentin¹⁷. The remineralization concept involves systematic approach to caries removal end point determination especially till the transparent zone within the inner carious dentin followed by the adhesive restoration¹⁶. This may be attributed to the presence of sound collagen structure and vital odontoblastic process¹⁸.

In the Papacarie Duo™ group, two patterns of residual dentin were observed. The first pattern revealed an amorphous layer indicative of smear layer and obliterated dentinal tubules, in accordance to previous studies^{2, 19, 20}. The second presented a regular surface with patent dentinal tubules and minimal smear layer was discerned². Whereas in case of Carie Care™ micrographs, it was observed that the dentinal tubules were open with some residual smear layer and fibrous structure inside the tubules. Another observation was the

Figure – 1 Scanning Electron Micrograph of dentin surface in Group I (x5000, 3µm) revealed amorphous layer indicative of smear layer and obliterated dentinal tubules

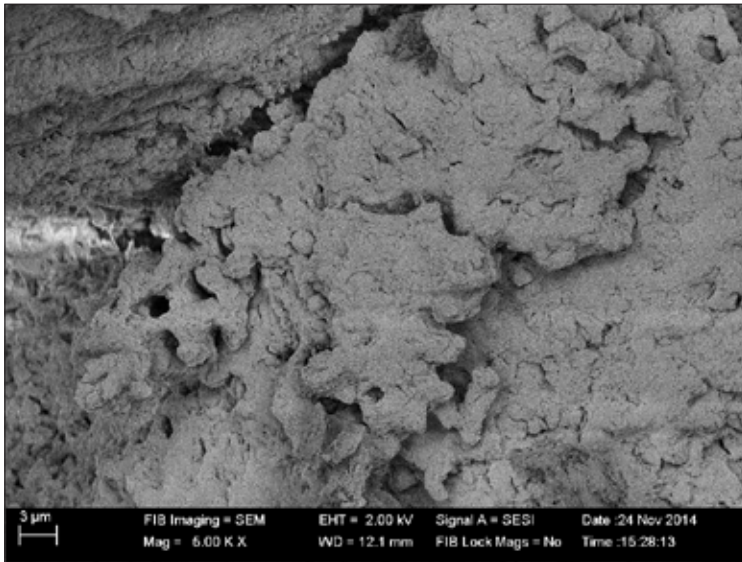


Figure – 2 Scanning Electron Micrograph of dentin surface in Group I (x10,000, 2µm) indicating patent dentinal tubules

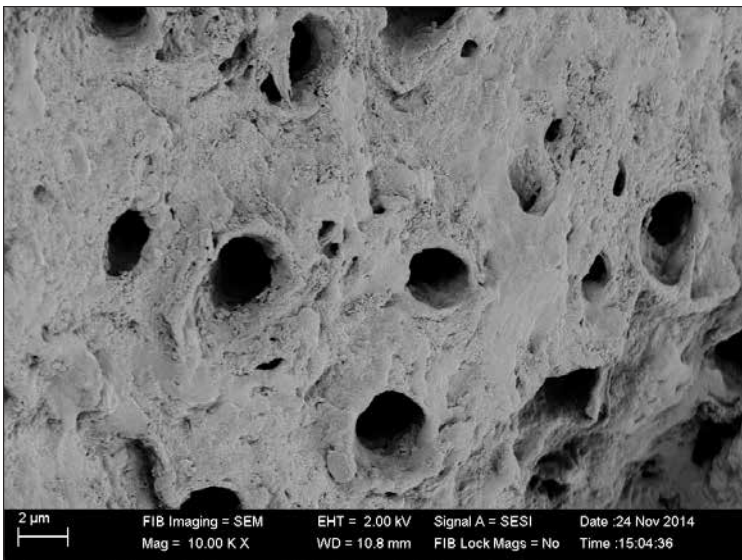


Figure – 3 Scanning Electron Micrograph of dentin surface in Group II (x10,000, 1µm) revealed intertubular microporosity with open dentinal tubules and minimal smear layer

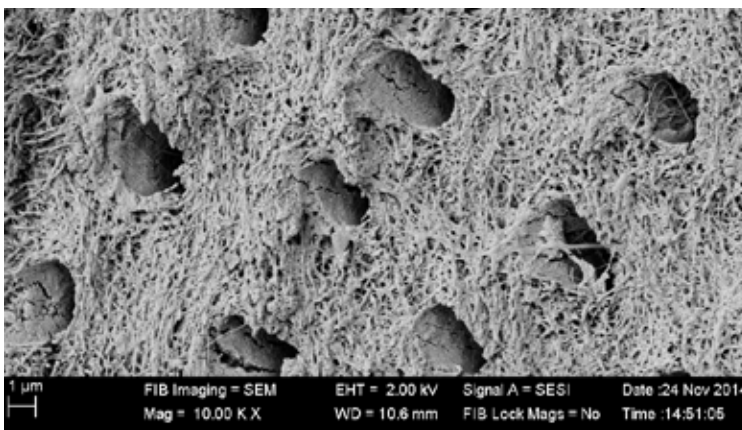


Figure – 4 Scanning Electron Micrograph of dentin surface in Group II (x10,000, 1µm) demonstrated fibrous structure inside the tubules and cross banded collagen fibrils

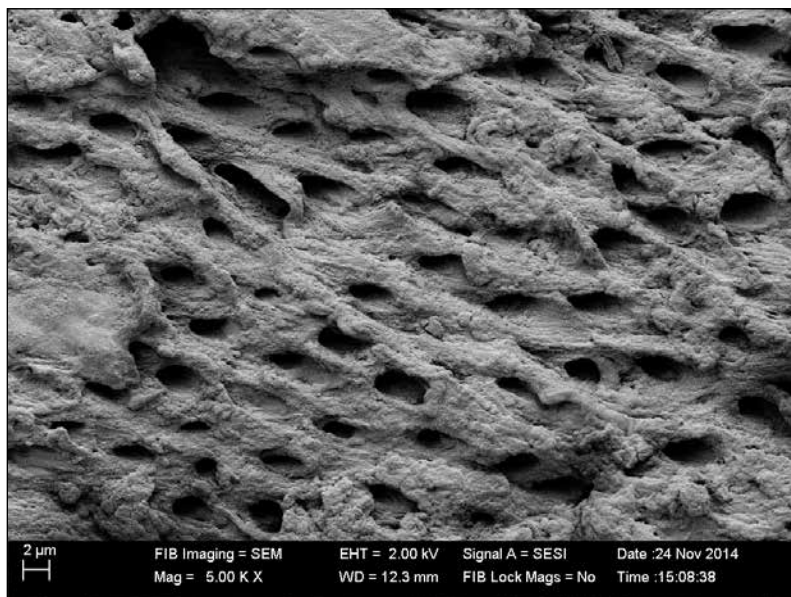
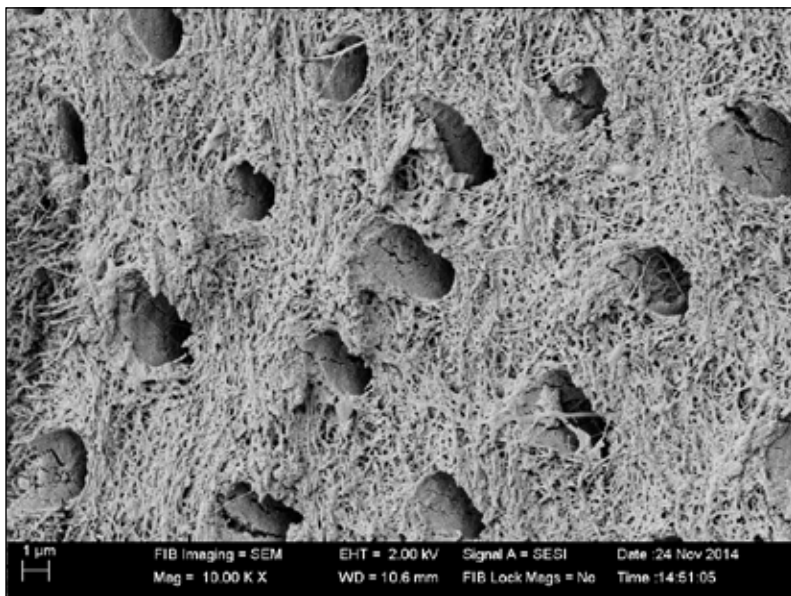


Figure – 5 Scanning Electron Micrograph of dentin surface in Group II (x5000, 2µm) with occluded dentinal tubules



presence of some fibrous structures in the dentinal tubules and the circular orientation of the peritubular collagen network suggestive of residual mineral deposit²¹.

In the present study the fibrous structure inside the tubules were appreciated in the specimens treated with the Carie Care™ representative of mineral deposit. The presence of mineral in the tubules is a well-known phenomenon that leads to formation of acid resistant transparent dentin, due to occlusion of the tubules which acts as a barrier to penetration of various irritants^{1, 22, 23}. Moreover the presence of crossbanded collagen in the inner dentin is believed to be essential in the remineralization process since apatite crystals can attach to these collagen molecules^{1, 24, 25}. Similar findings of

mineral crystals along with cross banded collagen in the peritubular and the intertubular dentin was reported in the specimens treated with Carie Care which confirms the removal of the caries infected dentin and approaching the transparent zone of the caries affected dentin approaching the transparent zone of caries²³.

According to the literature, peritubular dentin does not induce significant changes in overall mechanical properties of the dentin, and dentin properties are largely dependent on the properties of the intertubular dentin. Similarly, it is likely that the intratubular mineral deposits do not contribute significantly to the mechanical properties of the dentin^{23, 26, 27}. Moreover, in some instances, mineralization appears to increase substantially in caries affected dentin, and such an increase can be accounted for only if the intertubular dentin becomes hypermineralized²³. Similar results have also been reported for primary carious dentin²⁸.

The presence of open dentinal tubules in chemo-mechanical caries removal with various CMCR agents is attributed to the initial high pH of the gel and the mechanical preparation technique^{29, 30}. This may be due to the presence of chloramine, a component of the CMCR agents which resulted in the opening of dentinal tubules in the outer layer of carious dentin. Chloramine may interact with both collagen and other proteins, facilitating dissociation of the collagen fibre structure³⁰. It decomposed degenerated collagen, which caused selective softening of the dentin consistently removing the smear layer and exposing the dentinal tubules of carious dentin¹⁵. The essential therapeutic oils which is one of the component of the Carie Care is rich in phenolic compounds namely eugenol and eugenol derivatives which are precursors of flavones, isoflavones and flavonoids. They have antioxidant, anti-inflammatory, DNA-protective, analgesic and antimicrobial properties³¹. It will provide the further protection to the residual dentin.

CONCLUSION

Scanning electron microscopy analysis revealed a difference in ultramorphology of residual dentin in primary teeth treated with the two CMCR agents. The specimens treated with Carie Care™ demonstrated the presence of the cross banding of peritubular and intertubular dentin representative of transparent zone of the caries affected dentin. Our finding supports the concept of obtaining sound dentin and therefore should provide a better bonding surface morphology to adhesive restorative material.

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